CARBOHYDRATE-BINDING PROTEINS FROM PLANT CELL WALLS AND THEIR POSSIBLE INVOLVEMENT IN EXTENSION GROWTH

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1. Introduction

Various plant tissues have been shown to contain proteins and glycoproteins which can bind carbohydrates with a high affinity and structural specifity. These lectins (or phytohemagglutinins) have recently become subject of growing interest in many laboratories, since they represent good tools for the study of cell surface carbohydrate groups. However, their physiological role in plants is completely unknown [1,2].

It has become clear during the past years that plant cell walls contain glycoproteins besides pectin, hemicelluloses and cellulose [3,4]. Although a great portion of the glycoprotein is regarded as covalently linked to the carbohydrates, some protein can be isolated from the walls by means of salt solutions. It has been considered that this fraction is representative of a precursor of the covalently bound glycoprotein; however the evidence obtained from pulse-chase experiments is not fully conclusive [5]. After feeding [^{14}C] proline to growing mung bean hypocotyl segments up to half of the radioactivity incorporated as proline and hydroxyproline into the wall proteins can be extracted with chaotropic agents [6]. This result suggested that the non-covalently bound glycoprotein constitutes a much greater portion of cell wall protein than had previously been thought.

This paper presents results to show that noncovalently bound protein fractions from growing as well as non-growing mung bean hypocotyl cell walls exhibit lectin properties. As the binding potential is strongly diminished towards more acidic pH-values, it is suggested that the cell wall lectins are involved in plant cell extension growth.

2. Material and methods

2.1. *Preparation of walls*

2.1.1. Method A

Two grams of growing upper or nongrowing lower 8 mm segments excised from the same dark grown mung bean seedlings were exhaustively ground using a great cold mortar and pestle with 1.5 ml of buffer (0.6 M sucrose in 0.01 M potassium phosphate, pH 6.0). After addition of 6 ml of the same buffer but containing 0.3 M sucrose, walls were harvested at 500 *g* for 5 min and washed four times with 0.5% (w/v) sodium dodecyl sulphate (20°C) followed by four washings with cold water.

2.1.2. Method B

Alternatively, the tissue was ground according to ref. [7] in the same manner but with 0.5% (w/v) Lensodel (= Nonidet P 40, Shell Chem. Comp.) instead of buffer followed by 4 washings with cold water.

2.2. *Extraction*

The walls were extracted with 4 ml of cold 0.5 M potassium phosphate (pH 7.1, 0.2% β-mercaptoethanol) by stirring for 30 min and washed with 3 ml of the same buffer. This was followed by a second extraction with 0.05 M EDTA in water containing 0.06% Triton-X-100 including sonication for 20 sec (Braun Sonic 300). In the case of total tissue grinding and washing was performed directly in the above 0.5 M phosphate buffer.

Cytoplasmic supernatants were either sonicated or not and cleared at 50 000 *g* for 30 min. These supernatants, and all of the cell wall extracts were dialysed

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against several changes of the buffer used in the lectin assay and were made up to 10 ml. Protein in the extracts was determined according to ref. [8] using bovine serum albumin as a standard.

2.3. *Lectin assay*

The lectins were assayed by measuring their ability to bind and agglutinate red blood cells. Rabbit erythrocytes were trypsinized [9] and suspended in 0.05 M sodium–potassium phosphate (pH 7.4, 0.9% saline) so that a 1:10 dilution had an optical density of 0.8 measured at 620 nm, 1 cm cuvette, Zeiss Spectralphotometer PM Q II. Twelve standard serial 2-fold dilutions were made with 25 μl of extract using a Takásy microdiluter in disposable V-hole microtiter plates (Cooke Engineering Comp.) and 25 μl of the red blood cell suspension was added. The titers were read after 5 hr at room temperature. One hemagglutinating unit (HU) is defined as the reciprocal of the last dilution which was just still able to fully agglutinate all erythrocytes (no red dot visible). Two parallel dilution series were normally made on the same extract. If the HU values were different the results from both parallels are given.

For measuring agglutination at different pH-values the extracts were dialysed against unbuffered 0.9% saline and the erythrocytes suspended in the same solution. Dilution steps were than made with 25 μl of a solution containing 0.9% saline and made from a mixture (1 vol + 1 vol) of 0.05 M Na_2HPO_4 and K_2HPO_4 buffered with 0.05 M citric acid to the appropriate pH-value. As the first dilution hole was unbuffered, the lowest HU which we were able to read in this case was 2.

3. Results

A comparison of the agglutinating activity extracted from the total tissue with the extracts from cytoplasm and cell wall indicates that during the isolation of the two fractions, which includes several washing steps, no appreciable amount of lectin has been lost (table 1). Most of the activity is clearly associated with the cell

Table 1
Carbohydrate binding capacity of subcellular fractions from growing and nongrowing mung bean hypocotyl segments. The cell walls were prepared by Method A

	Growing segments			Nongrowing segments		
	Hemagglutinating activity			Hemagglutinating activity		
	Total [HU/25 μl extract*]	Specific [HU/μg protein]	[HU/100 μg cell wall**]	Total [HU/25 μl extract*]	Specific [HU/μg protein]	[HU/100 μg cell wall**]
Total tissue buffer extract	128	7	–	128/256	13/26	–
Cytoplasm sonicated	8/16	0.7/1.3	–	1	0.2	–
Cytoplasm Not sonicated	2	0.2	–	0	0	–
Cell wall buffer extract	64	136	93	64/128	160/319	151/303
Cell wall EDTA extract	8	30	12	16/32	60/120	38/76

* 25 μl of extract are equivalent to 5 mg fresh tissue.
** Dry weight of the cell wall residue after extraction.

Table 2
Carbohydrate binding capacity of buffer extracts from total tissue and cell walls prepared by two different methods from 2 g of nongrowing segments

		Protein [μg/25 μl extract*]	Hemagglutinating activity total [HU/25 μl extract*]	specific [HU/μg protein]
Total tissue		11.5	128	11
Walls prepared by:	Method A	0.3	128/256	426/853
	Method B	5.3	256/512	48/97

* 25 μl of extract are equivalent to 5 mg fresh tissue.

walls, especially in the case of the non-growing segments. The higher titer in the sonicated half of the cytoplasm indicates that the part not associated with the walls is not soluble in the cytosol but has to be released from membrane lined cell organelles. The association of the agglutinating activity with the cell wall appears increasingly pronounced if the specific activity of the wall extracts is compared with that for cytoplasm and total tissue. This result implies that no unspecific absorption of cytoplasmic protein took place during isolation of the walls.

To demonstrate this fact further we compared the specific activities of buffer extracts from carefully washed cell walls (Method A) with those from walls prepared by Method B, which gives walls with a relatively high protein content (table 2). Although the total agglutinating activity is similar we found a striking difference in specific activities; this was presumably due to the absorption of non-cell wall proteins when Method B was used.

In addition to the buffer a dilute solution of EDTA containing small concentrations of the detergent Triton-X-100 resulted in extraction of another fraction which also exhibits lectin properties (table 1). We first thought that this might represent an undissolved part of the buffer soluble fraction. However, preliminary results from studies performed at present in our laboratory in collaboration with D. J. Bowles indicate that both fractions have marked differences in respect to their carobhydrate group specifity. We therefore feel that the mung bean cell walls may contain several lectins.

The binding mechanism of lectin to carbohydrate groups closely resembles the binding between an enzyme and its substrate [1,2]. Therefore it is understandable that the agglutinating activity of lectins is strongly pH-dependent [1]. This pH-dependence is also demonstrated for the cell wall lectin from mung beans by the data given in table 3. The binding capacity is obviously lower towards more acidic pH-values.

Table 3
Influence of the pH-value on the carbohydrate binding capacity

pH	Hemagglutinating Activity [HU/25 μl extract] Expt. I	Expt. II
7.4	512	64
7.0	–	8
6.4	128	2
6.0	–	<2
5.5	<2	<2

Buffer extracts (see table 1) of cell walls prepared by Method A from nongrowing segments were used as lectins. Experiments I and II were performed independently with two samples exhibiting different initial hemagglutinating activity.

4. Discussion

It is generally considered that at least the wall material of hemicellulosic and pectic nature is carried via Golgi-derived vesicles towards the wall. Glycoproteins characterized by a high content of hydroxyproline and of similar composition as the cell wall protein constituents appear to occure in such vesicles also [3–5]. If these glycoproteins are lectins, which also may occur in membrane organelles (table 1), they could already be bound in the vesicles to cell wall polysaccharides owing that chemical structure to which their binding site is specific. The fact that the wall lectins can agglutinate erythrocytes implies that they may have more than one binding site per molecule. After fusion of the vesicles with the plasma membrane, complexes between lectins and polysaccharides would be extruded into the wall. This would enable them to find their 'right' place in the organized wall architecture.

In addition to a possible role in polysaccharide transport the wall lectins also offer a potential means to envisage mechanical properties of the wall. Cell enlargement involves a stretching of the relatively rigid wall. The forces which hold the wall constituents together have been envisaged partly as covalent links. However, serious difficulties must be overcome if the mechanism of wall extension is to be explained on that basis alone (e.g. refs. [10,11]. It has been suggested [12] that also noncovalent bonds may be involved in the regulation of wall extension, but up to the present time the exact nature of wall components with respective properties is unknown.

We suggest that the cell wall lectins could play such a role, as they are highly specific for polysaccharides and exhibit a sufficient bond energy [1,2]. Their function in the wall could take the form of a non-covalent 'glueing' substance. In this context, it is interesting that extracts from nongrowing walls exhibit higher binding capacity (table 1). Alternatively, if it is valid that the salt extractable glycoprotein fractions are precursors of the covalently-bound ones [5], the lectins may be partially linked covalently to certain polysaccharides, and simultaneously form a connection to other polysaccharides that possess the lectin specific sugar groups.

Many aspects of growth hormone action can be explained on the basis that lectins are responsible for growth-regulating noncovalent linkages within the cell wall. There is increasing evidence [10,11,13–15] that auxins stimulate the production of protons in the cell membrane which then enter the wall as a 'wall-loosening' factor. The observed lowering of the binding capacity of the cell wall would become mechanically more flexible under the influence of auxin and an increase in extension growth could result.

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